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Insulin stimulation assays

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An abbreviated version of this protocol was published in Science Advances in Jun 2022

Development of a physiological insulin resistance model in human stem cell–derived adipocytes

DOI: 10.1126/sciadv.abn7298

Detailed protocol

Insulin sensitization and stimulation assays of hPSC-adipocytes

Materials and reagents

1. DMEM, low glucose, pyruvate (Thermo Fisher 11885)
2. DMEM, no glucose, no glutamine, no phenol red (Thermo Fisher A1443001)
3. Insulin solution human (Sigma-Aldrich I9278)
4. Cellastim - Recombinant human albumin (InVitria 777HSA017S)
5. Penicillin-Streptomycin (Thermo Fisher 15140122)

Sensitization medium:

a. DMEM, low glucose, pyruvate (Thermo Fisher 11885)
b. 100pM Insulin solution human (Sigma-Aldrich I9278)
c. 200ug/mL Cellastim - Recombinant human albumin (InVitria 777HSA017S)
d. 1% Penicillin-Streptomycin (Thermo Fisher 15140122)

Assay medium:

a. DMEM, no glucose, no glutamine, no phenol red (Thermo Fisher A1443001)

Procedure

Insulin sensitization

1. Differentiate hPSCs into adipocytes as previously described.
2. Completely remove all medium.
3. Add 0.25mL/cm^2 sensitization medium.
4. After 48 hours and 96 hours repeat step 2-3.
5. 120 hours after the start of sensitization, 24 hours after the last medium change, cells are sensitized and ready for functional assays.

Note: For hyperinsulinemia-induced insulin resistance sensitization medium can be modified to include 3nM insulin.

Insulin stimulation assays

1. After insulin sensitization, hPSC-adipocytes can be prepared for functional assays.
2. Fully remove medium and rinse cells with assay medium.
3. Fully remove medium and add 0.25mL/cm^2 assay medium to the cells.
4. Place cells in an incubator at 37°C for 30 minutes.
5. Fully remove medium.
6. Add assay medium ±insulin as appropriate for the assay.
7. Place cells in an incubator at 37°C for the appropriate time for the assay.
8. Harvest the cells.

Note: For the original publication step 7 was 10 minutes for AKT2 phosphorylation and 40 minutes for glucose uptake assays.
Insulin concentrations can be varied as desired.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Friesen, M. and Jaenisch, R. (2022). Insulin stimulation assays. Bio-protocol Preprint. bio-protocol.org/prep1786.
2. Friesen, M., Khalil, A. S., Barrasa, M. I., Jeppesen, J. F., Mooney, D. J. and Jaenisch, R.(2022). Development of a physiological insulin resistance model in human stem cell–derived adipocytes. Science Advances 8(24). DOI: 10.1126/sciadv.abn7298